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ACKNOWLEDGMENTS

The authors thank Dr. Ragnar Ryhage and Yvonne Mårde for the mass spectrometric analysis.

GLC Determination of Aprindine in Human Plasma Using a Nitrogen–Phosphorus Flame-Ionization Detector

J. F. NASH ˣ and R. H. CARMICHAEL

Received November 30, 1979, from *Lilly Research Laboratories, Indianapolis, IN 46206.* Accepted for publication April 1, 1980.

Abstract □ A procedure for the determination of aprindine in human plasma was developed. After the addition of *N,N*-diethyl-*N'*-(1,2,3,4-tetrahydro-2-naphthyl)-*N'*-phenyl-1,3-propanediamine as an internal standard, the plasma was buffered to pH 8.0, and the drug and the internal standard were extracted into ethyl acetate–hexane (9:1 v/v). The compounds then were extracted from the organic phase into 0.02 *N* HCl. The acidic solution was made basic with 0.2 *M* tribasic sodium phosphate, and aprindine and the internal standard were extracted into a small volume of hexane. The compounds were analyzed by GLC using a nitrogen–phosphorus flame-ionization detector. The drug concentration and instrument response were linear for 0.10–1.00 μg of aprindine/ml, the slope was 1.1416 (0.0141), the *y* intercept was 0.0096 ± 0.0082, and the correlation coefficient was 0.99960 ± 0.00002. The sensitivity of the method was 0.02 μg of aprindine/ml. The within-day coefficient of variation was 9.50, 3.10, 3.14, and 2.21% for 0.05, 0.20, 0.40, and 0.80 μg of aprindine/ml, respectively. The between-day coefficient of variation was 17.4, 3.40, 2.07, and 1.54% at the same concentrations. Total precision values of 19.9, 4.60, 4.26, and 2.69% were obtained. The overall relative error of the method was +1.33, +2.00, −0.07, and +0.25% at these concentrations.

Keyphrases □ Aprindine—GLC analysis in human plasma using nitrogen–phosphorus flame-ionization detection □ GLC—analysis, aprindine, nitrogen–phosphorus flame-ionization detection, human plasma □ Antiarrhythmic agents—aprindine, GLC analysis using nitrogen–phosphorus flame-ionization detection, human plasma

Aprindine[1] (*N,N*-diethyl-*N'*-2-indanyl-*N'*-phenyl-1,3-propanediamine), an oral, long-acting, antidysrhythmic agent, has been used in Europe since the early 1970's. A sensitive (0.1 μg/ml), specific, and precise (coefficient of variation 5%) GLC method was developed (1) and then modified (2) that satisfactorily monitors plasma aprindine levels in patients. Lagerström and Persson (3) reported a sensitive (0.5 μg/ml) high-performance liquid chromatographic assay for monitoring plasma aprindine levels. These methods appear to be suitable for monitoring plasma aprindine levels of ≥0.5 μg/ml. However, for pharmacokinetic and bioavailability studies following unit doses of 10, 25, or 50 mg of aprindine, a more sensitive method was necessary.

This report describes a GLC method using a dual nitrogen–phosphorus flame-ionization detector that optimizes the sensitivity and specificity of the measurement of aprindine and still retains the precision of the assay.

[1] Christiaens Pharmaceutical Co., Brussels, Belgium.

EXPERIMENTAL

Chemicals and Reagents—Ethyl acetate[2], hexane[2], and methanol[2] were distilled in glass; all other chemicals and reagents were analytical reagent grade. Aprindine hydrochloride[3], *N,N*-diethyl-*N'*-(1,2,3,4-tetrahydro-2-naphthyl)-*N'*-phenyl-1,3-propanediamine hydrochloride[4], and [*N-phenyl*-1-^{14}C]aprindine hydrochloride[5] with a specific activity of 29.4 μCi/mg were synthesized.

The aprindine standard solution (10 μg/ml) was prepared by weighing accurately 1.12 mg of aprindine hydrochloride into a 100-ml volumetric flask and diluting the solution to volume with distilled water.

The *N,N*-diethyl-*N'*-(1,2,3,4-tetrahydro-2-naphthyl)-*N'*-phenyl-1,3-propanediamine solution (the internal standard) (5 μg/ml) was prepared by weighing accurately 1.11 mg of the hydrochloride salt of the internal standard into a 200-ml volumetric flask and diluting the solution to volume with distilled water.

GLC System—Table I summarizes the GLC equipment, materials, and conditions for the identification and analysis of aprindine in plasma. Disposable 15-ml centrifuge tubes with plastic-lined screw caps[6] were used throughout the extraction.

Procedure—A plasma calibration curve was prepared by pipetting 1.0 ml of fresh, human, drug-free plasma into each of five disposable 15-ml centrifuge tubes and adding 250 μl of the internal standard solution to give a concentration of 1.25 μg/ml. The first tube served as the blank. Ten microliters of the aprindine standard solution was added to the second tube, 25 μl was added to the third tube, 50 μl was added to the fourth tube, and 100 μl was added to the fifth tube. These plasma standards contained aprindine concentrations of 0.00, 0.10, 0.25, 0.50, and 1.00 μg/ml, respectively.

Borate buffer USP, 0.5 ml, was mixed thoroughly with the plasma standards. Then 10 ml of ethyl acetate–hexane (9:1 v/v) was shaken with each tube of plasma standard for 2 min, and the phases were separated by centrifugation. Approximately 8 ml of the supernate was pipetted into a clean 15-ml centrifuge tube, with care taken not to remove any of the aqueous phase. The compounds were extracted into 5 ml of 0.02 *N* HCl, the contents of the tube were centrifuged, and the organic layer was discarded. The aqueous solution was made basic by mixing with 1.5 ml of 0.2 *M* tribasic sodium phosphate, the drug and the internal standard were extracted into 0.3 ml of hexane, and the phases were separated again by centrifugation. Two microliters of the hexane solution was injected onto the chromatographic column.

When assaying plasma samples, a 1.0-ml sample was placed into a 15-ml disposable centrifuge tube and 250 μl of the internal standard solution was added. After 0.5 ml of borate buffer was added, the plasma samples were handled as were the calibration curve samples.

A least-squares line was prepared by analysis of the calibration curve of the peak height ratio of aprindine to the internal standard *versus*

[2] Burdick & Jackson, Muskegon, Mich.
[3] Fibocil, Eli Lilly and Co., Indianapolis, Ind.
[4] Eli Lilly and Co., Indianapolis, Ind.
[5] New England Nuclear, Boston, Mass.
[6] Corning Glass Works, Corning, N.Y.

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Table I—Conditions for the GLC Identification and Analysis of Aprindine and the Internal Standard [a] in Plasma Extracts

Instrument	Gas Chromatograph[b]	Gas Chromatograph–Mass Spectrograph[c]
Detector	Nitrogen–phosphorus flame-ionization detector[b]	Mass spectrograph[c]
Recorder	Model 7127A, 1 mv[b]	Model 6000[c]
Packing	3.8% W-98[d]	2% OV-7[d]
Support	Chromosorb G-HP[d] (80–100 mesh)	Chromosorb W-HP[d] (80–100 mesh)
Column length, cm	91.4	91.4
Column inner diameter, mm	3	2
Gases and flow rates, ml/min		
Carrier	Helium, 60	Source pressure
Flame	Hydrogen, 60	800–1000 μm
Support	Oxygen, 240	Methane, 18
Oven temperature	255°	240°
Injection port temperature	250°	270°
Detector temperature	250°	180°
Attenuation	1×10^{-9}	—
Chart speed, cm/min	0.635	—
Ionization energy, ev	—	110

[a] *N,N*-Diethyl-*N'*-(1,2,3,4-tetrahydro-2-naphthyl)-*N'*-phenyl-1,3-propanediamine. [b] HP 5700A, Hewlett-Packard Co., Avondale, Pa. [c] Model 3200, Finnigan Corp., Sunnyvale, Calif. [d] Ohio Valley Specialty Chemical Co., Marietta, Ohio.

concentration. The concentration of aprindine in the plasma samples was determined from their peak height ratios and the least-squares line.

RESULTS AND DISCUSSION

With [*N-phenyl*-1-^{14}C]aprindine hydrochloride, it was found that ~80% of the aprindine in spiked plasma samples was recovered following the described procedure (Table II).

During the development of the method, aprindine adsorbed irreversibly to the glass surface during the evaporation of hexane in the final concentration step. Silylation of the dry-down tubes and/or addition of 20 μl of dimethylformamide to the hexane only partially prevented the binding. Therefore, only a small volume of hexane was used as the collection solvent for the compounds. When the final hexane layer was not concentrated, the binding of aprindine to glass was prevented and the

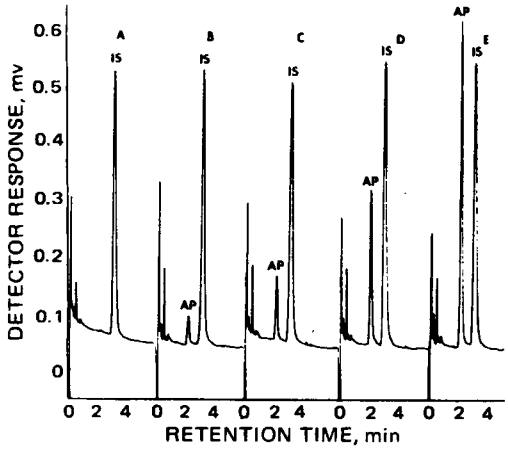

Figure 1—*Gas–liquid chromatograms (nitrogen–phosphorus detector) of a plasma blank with the internal standard (A) and plasma standards with aprindine at 0.10 (B), 0.25 (C), 0.50 (D), and 1.00 (E) μg/ml. Key: AP, aprindine; and IS, internal standard. The temperature was 255°. The 91.4-cm column was packed with 3.8% W-98 on Chromosorb G (HP). The attenuation was 64. Two microliters was injected.*

Table II—Analytical Recovery of [*N-phenyl*-1-^{14}C]Aprindine Added to Plasma

Concentration Added[a], μg/ml	Recovery[b], %	*CV*, %
0.5	82.6	7.0
2.0	80.0	3.3

[a] As [*N-phenyl*-1-^{14}C]aprindine hydrochloride; five replicates. [b] Found in hexane phase after analyzing samples by the reported procedure, correcting for volume transferred, and comparing to the same unextracted concentration of [*N-phenyl*-1-^{14}C]aprindine in hexane.

Table III—Standard Curves for Aprindine in Plasma [a]

Aprindine[b] Added to Plasma, μg/ml	Peak Height Ratio, Aprindine to Internal Standard	Aprindine[c] Found in Plasma, μg/ml
0.10	0.1197 (0.0086)	0.113 (0.0003)
0.25	0.2628 (0.0086)	0.238 (0.0013)
0.50	0.5536 (0.0124)	0.493 (0.0132)
1.00	1.1376 (0.0167)	1.005 (0.0036)

[a] Linear regression equation: $y = 1.1416\ (0.0141)x - 0.0096\ (0.0082)$; $r = 0.99960$ (0.00002). [b] As aprindine hydrochloride. [c] Mean (±*SD*) of three replicate curves assayed on 3 different days by the same analyst.

reproducibility of the method was improved greatly while suitable sensitivity was maintained.

Table III shows that the data from three typical plasma calibration curves were linear over at least a 10-fold concentration range with an intercept of near zero. The small standard deviations of the data used to prepare the least-squares lines and the correlation coefficients indicate the reproducibility of the method.

Figure 1 shows typical chromatograms of blank human plasma and a plasma calibration curve obtained by using the nitrogen–phosphorus flame-ionization detector. Chromatograms of blank human plasma and a plasma calibration curve obtained using a flame-ionization detector are given in Fig. 2. Comparison of the nitrogen–phosphorus chromatograms to the flame-ionization chromatograms shows that the former detector was 16 times more sensitive under the conditions stated for the detection of aprindine (calculated by noting four times the peak height of aprindine standards at four times the instrument attenuation). It was necessary to acid wash glassware and to optimize the nitrogen–phosphorus detector daily, as reported by Pierce *et al.* (4), to maintain good reproducibility and sensitivity.

The major metabolite of aprindine, desethylaprindine (1), eluted at ~60 sec, which separated it from the aprindine (150 sec) and the internal standard (190 sec). When humans were given therapeutic doses of aprindine hydrochloride, desethylaprindine was never seen in their plasma; however, the metabolite was seen and quantitated in dog plasma after the dogs were dosed with the drug[7]. Standard vacuum containers[8], used to collect blood samples, produced no interference in the determination of aprindine by this method.

Gas–liquid chromatograms–mass spectrograms (methane as positive ion for chemical ionization) of plasma extracts were similar to solid-probe

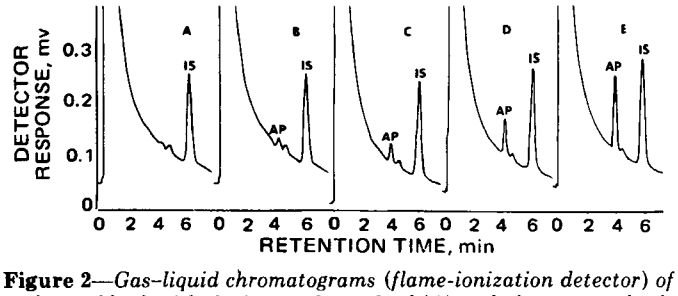

Figure 2—*Gas–liquid chromatograms (flame-ionization detector) of a plasma blank with the internal standard (A) and plasma standards with aprindine at 0.10 (B), 0.25 (C), 0.50 (D), and 1.00 (E) μg/ml. Key: AP, aprindine; and IS, internal standard. The temperature was 240°. The 91.4-cm column was packed with 3.8% W-98 on Chromosorb C (HP). The attenuation was 16. Two microliters was injected.*

[7] J. F. Nash and R. H. Carmichael, Eli Lilly and Co., Indianapolis, Ind., unpublished data.

[8] Vacutainer, Becton-Dickinson Co., Rutherford, N.J.

Table IV—Precision and Accuracy in Assay of Added Aprindine to Human Plasma

	In Vitro Pools, μg/ml			
	0.050	0.200	0.400	0.800
Number of replicates	5	5	5	5
Mean, μg/ml	0.048	0.200	0.390	0.796
CV, %	9.32	3.54	4.80	2.45
Number of replicates	5	5	5	5
Mean, μg/ml	0.046	0.202	0.400	0.796
CV, %	11.91	2.21	1.77	1.43
Number of replicates	5	5	5	5
Mean, μg/ml	0.058	0.210	0.408	0.814
CV, %	7.71	3.37	2.05	2.55
Overall precision				
Between-day *CV*, %	17.4	3.40	2.07	1.54
Within-day *CV*, %	9.50	3.10	3.14	2.21
Total *CV*, %	19.9	4.60	4.26	2.69
Overall accuracy				
Mean, μg/ml	0.051	0.204	0.399	0.802
Total relative error, %	+1.33	+2.00	−0.07	+0.25

spectrograms of aprindine. The protonated $[M + H]^+$ ion at *m/e* 323 and fragmentation ions at *m/e* 113, 117, and 207 confirmed the presence and integrity of aprindine in the plasma of subjects administered the drug.

The accuracy and precision of the method were determined by preparing four plasma pools of known aprindine concentrations. The same analyst assayed five replicates from each pool on each of 3 days (Table IV). Good reproducibility was indicated by the within-day coefficients of variation of 9.50, 3.10, 3.14, and 2.21% for 0.05, 0.20, 0.40, and 0.80 μg of aprindine/ml, respectively, as well as by the between-day variations of 17.4, 3.40, 2.07, and 1.54% at the same concentrations. The total coefficients of variation were 19.9, 4.60, 4.26, and 2.69%, respectively. The overall relative errors at 0.05, 0.20, 0.40, and 0.80 μg of aprindine/ml were +1.33, +2.00, −0.07, and +0.25%, respectively.

Because of the specificity, sensitivity, precision, and accuracy of the reported method for the determination of aprindine in plasma, bioavailability and pharmacokinetic studies of aprindine can be undertaken at therapeutic doses. These studies will be reported later.

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ACKNOWLEDGMENTS

The authors thank Dr. R. L. Wolen for the GLC–mass spectrometric analyses, Mr. W. E. Legan for help in development of the method and analyses of the plasma samples, and Mrs. K. Z. Farid for helpful suggestions in method development.

NOTES

Measurement of Pilocarpine and Its Degradation Products by High-Performance Liquid Chromatography

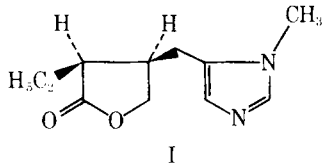
JAMES J. O'DONNELL ˣ, ROBERT SANDMAN, and MICHAEL V. DRAKE

Received January 22, 1980, from the *Department of Ophthalmology, University of California Medical Center, San Francisco, CA 94143.* Accepted for publication March 21, 1980.

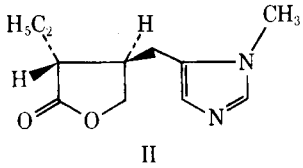
Abstract □ An assay method for pilocarpine using reversed-phase high-performance liquid chromatography is presented. This method also measures isopilocarpine, the stereoisomer of pilocarpine, and the degradation products pilocarpic acid and isopilocarpic acid. Maximum sensitivity was obtained with optical absorbance at 216 nm.

Keyphrases □ Pilocarpine—reversed-phase high-performance liquid chromatographic analysis □ High-performance liquid chromatography—analysis, *pilocarpine*, isopilocarpine, pilocarpic acid, and isopilocarpic acid □ Ophthalmic drugs—pilocarpine, high-performance liquid chromatographic analysis

Pilocarpine (I) is a natural alkaloid used for the parasympathomimetic treatment of glaucoma. Isopilocarpine (II), the therapeutically inactive stereoisomer of pilocarpine, is present in nature and in commercial preparations of pilocarpine (1, 2). The degradation products,



I



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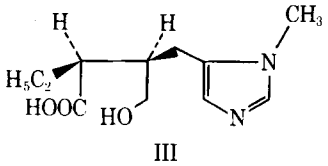
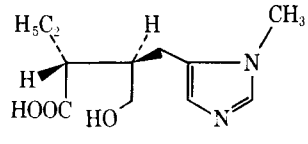


III



IV

pilocarpic acid (III) and isopilocarpic acid (IV), also are found in commercial products (3). This report presents an efficient and sensitive assay for I–IV using reversed-phase high-performance liquid chromatography (HPLC). This method is a modification of a procedure described recently (4).

EXPERIMENTAL

Pilocarpine hydrochloride USP[1] and isopilocarpine hydrochloride[2] were obtained commercially. Pilocarpic acid and isopilocarpic acid were prepared by hydrolysis of pilocarpine and isopilocarpine in 0.1 *N* NaOH. Isocratic reversed-phase chromatography was accomplished by pumping[3]

[1] Mallinckrodt, St. Louis, Mo.
[2] Aldrich, Milwaukee, Wis.
[3] Model 310 high-performance liquid chromatograph, Altex Scientific Inc., Berkeley, Calif.

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